The Disappearance of Immuno-Reactive Insulin in Anephric Man and the Concomitant Effect on Glucose, Cortisol and Growth Hormone Levels

G SNODGRASS, R O ROBINSON, K MASHITER, C S OGG, J S CAMERON, F G ELLIS and L STIMMLER
Guy's Hospital Medical School, London, United Kingdom

Impairment of carbohydrate tolerance in uraemic patients has been known for fifty years. This abnormality has been convincingly demonstrated following oral (Perkoff et al, 1958) and intravenous glucose loading (Horton et al, 1968) in such patients. This cannot be explained by a deficiency of insulin secretion, for plasma insulin levels have been shown to be relatively high and to remain elevated for an abnormal duration following oral (Tchobroutsky et al, 1965) or intravenous glucose loading (Hutchings et al, 1966). One explanation, currently favoured, is that of an increased tissue resistance to the normal effects of insulin (Cerletty & Engbring, 1967; Horton et al, 1968). It has been shown by Chamberlain and Stimmler (1967) that in normal subjects a significant difference exists (30%) between immunoreactive insulin (IRI) concentrations in renal venous and renal arterial blood. These workers assume an important role for the kidney for the degradation of insulin.

Using a different approach we have studied the disappearance of IRI in anephric subjects as compared with normal controls. We have also taken the opportunity to examine some aspects of function of the hypothalamic-pituitary-adrenal axis by measuring simultaneous changes in HGH and Cortisol levels during the same experimental situation.

METHODS

Fourteen normal volunteers (9 males and 5 females) and ten anephric patients (8 males and 2 females) were studied. The mean age of the normal controls was 35.5 years (range 24-63 years) and that of the anephric patients 32.4 years (range 20-56 years). The anephric patients were all having regular haemodialysis treatment for 14 hours twice weekly using Kiil dialysers with PT 300 cellulose membranes, at blood flows averaging 160 ml/min. Seven patients were studied immediately prior to dialysis and three studied two hours after cessation of treatment.

The study was begun after 14 hours of starvation in both normal and

anephric subjects. All subjects were rested for one hour prior to starting the test. The mean pre-dialysis plasma creatinine was 15 mg/100 ml (SD ± 5.6) for the anephric patients and the mean pre-dialysis blood urea was 177 (SD ± 36) mg/100 ml. Post-dialysis the mean blood urea was 55.4 (SD ± 25) mg/100 ml.

Fasting blood was taken for glucose, IRI, cortisol and human growth hormone (HGH) levels. Porcine insulin, 0.1 u/kg body weight was then given intravenously into the arm not being used for sampling. Further samples were obtained from an indwelling venous cannula in the controls and from the arterio-venous shunt in the anephric patients. Samples were then taken at $2\frac{1}{2}$ minute intervals for 25 minutes, then at 5 minute intervals for a further 25 minutes and subsequently at 10 minute intervals for 30 minutes. All the heparinised samples were kept on ice and separated in batches of five. Blood glucose was measured by the glucose oxidase method using a standard auto-analyser technique. Plasma IRI was estimated by the method of Morgan and Lazarow (1962). Plasma HGH was also measured by a double antibody method described by Hartog et al (1964). Plasma 11-hydroxycorticosteroids were estimated by the method of Mattingly (1962) with slight modifications.

RESULTS

Insulin

The plasma levels of IRI before and after administration of porcine insulin are shown for normal controls (Table I) and for the anephric patients (Table II). Fasting values were similar for the two groups. Five minutes after

Table I. The plasma IRI in 14 normal adult subjects following injection of porcine insulin 0.1 u/kg body weight

Subject	Minutes												
	0	5	7.5	10	12.5	15	17.5	20	25	30	35	40	45
1	4	910	305	182	120	94	65	49	29	21	13	9	7
2	4	397	310	199	152	132	72	49	30	10	10	10	10
3	6	240	229	188	131	88	70	58	35	21	13	13	13
4	2	460	363	163	107	89	72	57	39	30	21	12	9
5	4	415	260	151	69	64	41	30	18	11	9	5	4
6	0	300	240	48	57	67	54	41	14	4	3	1	0
7	0	700	425	245	183	135	119	91	60	40	-	-	-
8	20	745	500	378	200	130	85	40	25	60	30	25	20
9	13	1700	970	330	230	180	130	80	50	40	20	12	5
10	0	450	445	195	157	110	100	90	65	0	15	30	50
11	0	2980	1545	200	190	0	30	27	20	0	0	0	0
12	25	1510	500	475	370	190	135	105	100	65	15	35	55
13	0	-	700	580	400	190	150	90	45	65	80	0	7
14	15	980	837	470	330	200	163	120	67	43	34	23	21
Mean	7	867	519	260	163	119	90	66	42	28	19	13	15

Table II. The plasma IRI in 10 anephric patients following porcine insulin 0.1 u/kg body weight

Subject	Minutes												
	0	5	7.5	10	12.5	15	17.5	20	22.5	25	30	35	40
1	10	510	435	368	248	165	135	106	70	66	45	32	24
2	5	570	475	378	313	245	193	130	110	101	93	58	35
3	6	438	430	320	245	160	128	118	97	100	66	35	34
4	6	360	355	213	205	130	88	76	56	50	33	28	25
5	-	850	363	295	230	173	146	120	132	86	65	45	38
6	10	245	115	89	66	50	46	40	33	25	20	20	15
7	9	270	196	134	86	57	49	41	36	31	21	18	13
8	9	221	-	112	86	63	53	46	38	27	23	17	14
9	9	245	200	78	85	86	62	31	33	33	22	13	12
10	9	284	-	141	106	70	56	38	37	28	23	17	16
Mean	8	399	321	213	167	121	83	75	64	55	41	28	22

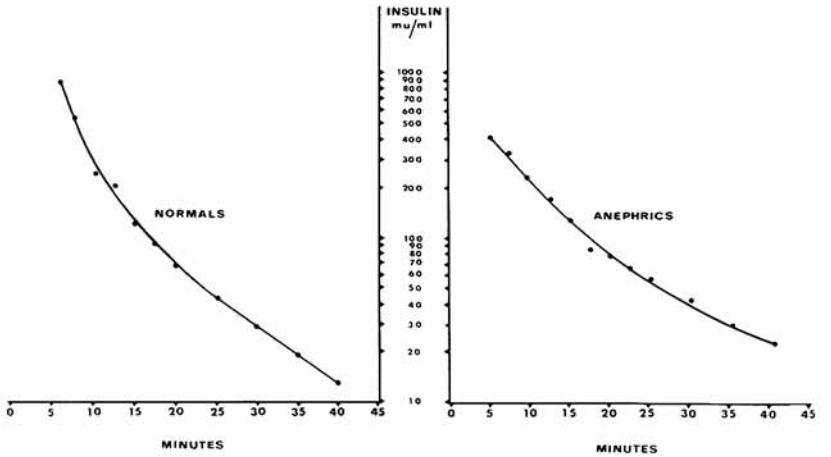

Figure 1. The mean IRI disappearance curve for normal controls and anephrics

intravenous insulin 0.1 u/kg body weight the levels were significantly lower in the anephric cases. Analysis of the IRI values from 5-20 minutes following insulin administration can be used to calculate its disappearance rate from plasma. The mean plasma disappearance rate of IRI for normals was found to be 17 $\pm$ 6% (SD) per minute, for anephrics it was 11 $\pm$ 1% (SD) per minute. The difference between the two groups was significant ($t = 4.37$, $p < 0.0005$). Although the mean 5 minute level of IRI is lower in the anephric group, it is the same at 15 minutes (Figure 1) and thereafter exceeds it. This is explained by the slower disappearance of IRI in anephric patients.

Glucose

There had already been a fall in the blood glucose levels 5 minutes after insulin administration in both groups. From 5-20 minutes after the insulin this fall was exponential (Figure 2). The mean rate of blood glucose disappearance for normals was 7.2 $\pm$ 2.6% (SD) per minute and 3.5 $\pm$ 1.4% (SD) per minute for anephric cases. This difference was significant ($t = 3.28$, $p < 0.0025$). The point at which the blood glucose reached its nadir was 27 $\pm$ 2.7 (SD) minutes for normals and 38.5 $\pm$ 14 minutes (SD) for the anephrics ($t = 2.53$, $p = 0.01$). The degree of the glucose fall was only slightly greater in the normal subjects than in the anephric patients. Expressed as a percentage of the fasting level this was 75% at the mean nadir for the controls and 63% at the mean nadir for glucose for the anephric cases.

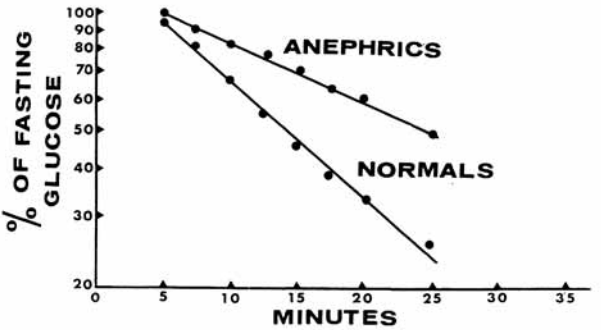

Figure 2. The mean glucose disappearance for normals and anephrics expressed as a percentage of the fasting level

Growth hormone

The mean plasma HGH values for the controls and for the anephrics are shown in Figure 3. The mean fasting levels were 5 $\pm$ 3 (SD) ng/ml for anephric patients and 4 $\pm$ 3 (SD) ng/ml for the controls. As can be seen these are very similar. There was a marked difference found for the peak values achieved, however. For normal subjects this was 36 $\pm$ 10 (SD) ng/ml while for the anephrics it was 53 $\pm$ 18 (SD) ng/ml. This difference was significant ($t = 2.28$, $p = 0.025$). Peak values also occurred earlier in the anephric group (39 $\pm$ 9 minutes) than in the normal controls (52 $\pm$ 16 minutes) and this difference was also significant ($t = 2.17$, $p = 0.025$).

Cortisol

The mean resting cortisol levels in the anephric group was 21 $\pm$ 5 (SD) μg/100 ml. This is rather high, but is compatible with the values obtained in a normal individual under conditions of stress. At 30 minutes the mean level

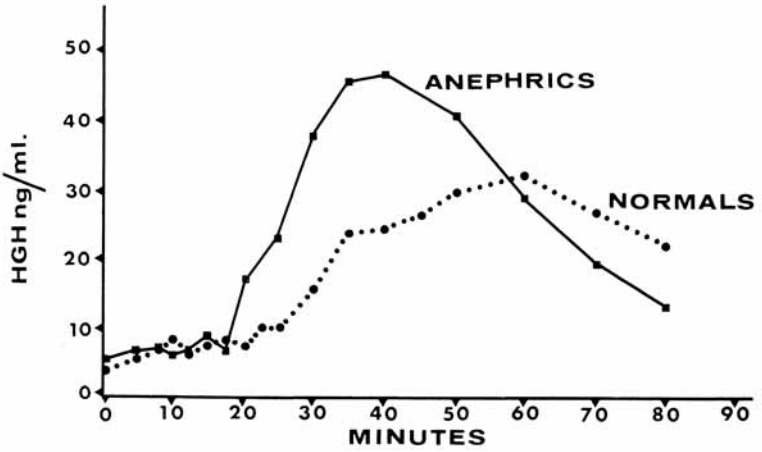

Figure 3. The mean HGH levels following intravenous insulin 0.1 u/kg in normal and anephric subjects

was 24 ± 11 (SD) μg/100 ml and at 60 minutes 33 ± 11 (SD) μg/100 ml. There was a satisfactory rise found, therefore, in response to the hypoglycaemic stress as reflected in the 60 minute values.

DISCUSSION

Although fasting levels of IRI were comparable for the two groups and equivalent doses of insulin had been given to all subjects according to body size, the 5 minute values were significantly lower in the anephric group ($t = 1.9$, $p < 0.05$). These low values can be partially explained by the increased plasma volumes of these patients, resulting in a greater initial dilution of the administered insulin. The anephric patients were anaemic (mean haemoglobin 7.3 ± 1.1 (SD) g/100 ml; PCV 22 ± 4 (SD) %). Several explanations have been offered for the slower disappearance of exogenously administered insulin in uraemia. These include a generalised tissue unresponsiveness to the action of insulin or a defect in its binding to sites of action in the tissues (Horton et al, 1968). If unresponsiveness of the tissues is the cause then it is not due to urea retention alone (Horton et al, 1968) and this is supported in the present study by finding no difference in the IRI disappearance rate for those cases studied before dialysis and those studied after dialysis. For the mean post-dialysis blood urea was 55.4 ± 25 mg/100 ml in these patients as compared with a mean level of 177 ± 36 (SD) during the same experimental situation for those tested before dialysis.

The important role of the kidney in man for insulin degradation has been shown by Chamberlain and Stimmler (1967). These authors have shown that 30% of the IRI passing through the kidneys is removed by them. Assuming

that 20% of the cardiac output passes through the kidneys it can be calculated that the renal rate of removal would amount to 6% per minute. From the data given it can be seen that the difference in the IRI disappearance rates between the two groups could therefore be explained by the absence of kidneys alone.

Within five minutes of insulin administration there was already an obvious effect on blood glucose levels. The rapidity of this effect is likely to be due to the high concentrations of circulating insulin present at this time, for as insulin levels fall so the rate of glucose disappearance falls until the nadir for glucose is reached. The later occurrence of the glucose nadir in anephric patients could be explained by the persistence of the exogenous insulin in their plasma for a longer period. That is to say, the insulin in the plasma continues to exert an effect on the blood glucose until it has disappeared. It can be calculated that when the IRI has fallen to 30 μu/ml then approximately 99% of the administered dose has disappeared from the plasma. The mean IRI at the nadir for glucose was 34 μu/ml for the normals and 29 μu/ml for the anephrics. An alternative explanation for the delayed glucose nadir in anephrics might be that the homeostatic mechanisms responsible for restoration of normal glucose levels are inadequate or delayed in their effect. The substantial and significantly early HGH peak levels, together with a highly adequate Cortisol response which is normal in timing and type, would make this suggestion unlikely. The lower rate of glucose disappearance in anephrics could be explained by the absence of kidneys which, under the influence of adequate amounts of circulating insulin, might be expected to take up a proportion of the glucose passing through them.

The significantly higher peak values of HGH in anephric patients would appear to be due to an increased secretion, rather than to a delay in its degradation, for a normal pattern of fall is seen subsequently. This fall has reached near basal levels at the 80 minute point of the test. The fasting values and those occurring up to 17.5 minutes after insulin are extremely similar for the two groups. Subsequent to this time a rapid rise in HGH occurs in the anephric group. The significantly earlier occurrence of the HGH peak in anephrics is not associated with an earlier glucose nadir, however. Protein-calorie malnutrition has been found to be associated with high fasting levels of HGH (Pimstone et al, 1967), but these patients averaged 55g protein/day, predominantly of animal origin, and their fasting HGH values were comparable with those of the normal controls.

The Cortisol response to hypoglycaemia showed that no defect of the hypothalamic-pituitary-adrenal axis was present. Adrenal reserve for Cortisol was also normal.

ACKNOWLEDGMENTS

The plasma Cortisol estimations were performed by Dr John Townsend and Dr Janice Went of the Department of Clinical Pathology at Guy's Hospital. The blood glucoses were also performed by this Department with the kind permission of Dr J Liddell.

REFERENCES

Cerletty, J. M., and Engbring, N. H. (1967) Annals of Internal Medicine, 66, 1097

Chamberlain, M. J., and Stimmler, L. (1967) Journal of Clinical Investigation, 46, 911

Hartog, M., Gaafar, M. A., Meisser, B. and Fraser, R. (1964) British Medical Journal, 2, 1229

Horton, E. S., Johnson, C. and Lebowitz, H. E. (1968) Annals of Internal Medicine, 68, 63

Hutchings, R. H., Hegstrom, R. M. and Scribner, B. H. (1966) Annals of Internal Medicine, 65, 275

Mattingly, D. (1962) Journal of Clinical Pathology, 15, 374

Morgan, C. R. and Lazarow, A. (1962) Proceedings of the Society of Experimental Biology (New York), 110, 29

Perkoff, G. T., Thomas, C. L., Newton, J. D., Sellman, J. C. and Tyler, F. H. (1958) Diabetes, 7, 375

Pimstone, B., Barbezat, G., Hansen, J. D. L. and Murray, P. (1967) Lancet, ii, 1333

Tchobroutsky, G., Collin de L'Hortet, G., Rosselin, G., Assan, R. and Derot, M. (1965) Diabetologia, 1, 101